FORM PTO-1390 (REV 5-93)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY DOCKET NO. 100564-00064
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		DATE: August 2, 2001
		U.S. APPLN. NO. (IF KNOWN, SEE 37 C.F.R. 1.5) Not Yet Assigned 09/889592

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/00877	3 February 2000	3 February 1999

TITLE OF INVENTION: PROTEIN WITH CELL PROLIFERATION AND CELL DIVISION MODULATING ACTIVITY AND DNA ENCODING SUCH PROTEIN

APPLICANT(S) FOR DO/EO/US: Ingvar M. FERBY, M. Angel Rodriguez NEBREDA

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371. (THE BASIC FILING FEE IS ATTACHED)

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures [35 U.S.C. 371(f)] at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☐ A proper demand for International Preliminary Amendment was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed [35 U.S.C. 371(c)(2)]
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☒ A translation of the International Application into English [35 U.S.C. 371(c)(2)].

7. ☒ Amendments to the claims of the International Application under PCT Article 19 [35 U.S.C. 371(c)(3)]
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 [35 U.S.C. 371(c)(3)].

9. ☒ An oath or declaration of the inventor(s) [35 U.S.C. 371(c)(4)].

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 [35 U.S.C. 371(c)(5)].

Items 11 - 16 below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.

12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information: ☒ PCT/RO/101; PCT/IPEA/409; 8 pages of sequence listing; 4 sheets of amended claims Drawings (5 sheets)

U.S. APPLN. NO. (IF KNOWN SEE 37 C.F.R. 1.5) 09/889592	INTERNATIONAL APPLICATION NO. PCT/EP00/00877	ATTORNEY DOCKET NO. 100564-00064 DATE: August 2, 2001

17. ☒ The following fees are submitted: **Basic National Fee [37 C.F.R. 1.492(a)(1)-(5)]:** Search Report has been prepared by the EPO or JPO.....$860.00 International preliminary examination fee paid to USPTO (37 C.F.R. 1.482).....$690.00 No international preliminary examination fee paid to USPTO (37 C.F.R. 1.482) but international search fee paid to USPTO [37 C.F.R. 1.445(a)(2)].....$710.00 Neither international preliminary examination fee (37 C.F.R. 1.482) or international search fee [37 C.F.R. 1.445(a)(2)] paid to USPTO.....$1,000.00 International preliminary examination fee paid to USPTO (37 C.F.R. 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....$ 100.00	CALCULATIONS	PTO USE ONLY
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date [37 C.F.R. 1.492(e)].	$ 0.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	17 - 20 =	0	X $ 18.00	$ 0.00	
Independent Claims	1 - 3 =	0	X $ 80.00	$ 0.00	
Multiple dependent claim(s) (if applicable)			+ $270.00	$ 0.00	
TOTAL OF ABOVE CALCULATIONS =				$ 860.00	
Reduction by one-half for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 C.F.R. 1.9, 1.27, 1.28). -				$ 0.00	
SUBTOTAL =				$ 860.00	
Processing fee of $130.00 for furnishing the English translation later the ☐ 20 ☐ 30 months from the earliest claimed priority date [37 C.F.R. 1.492(f)]. +				$ 0.00	
TOTAL NATIONAL FEE =				$ 860.00	
Fee for recording the enclosed assignment [37 C.F.R. 1.21(h)]. The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). $40.00 per property +				$ 40.00	
TOTAL FEES ENCLOSED =				$ 900.00	
				Amount to be refunded	$
				Charged	$

a. ☒ A check in the amount of $900.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 01-2300 in the amount of $ to cover the above fee. A duplicate copy of this sheet is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 01-2300.

NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive [37 C.F.R. 1.137(a) or (b)] must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
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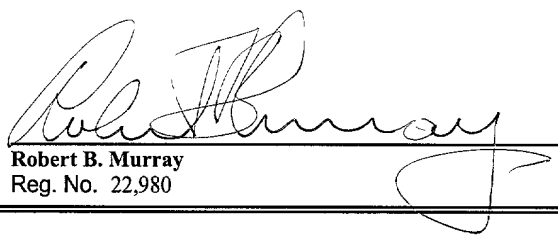

Robert B. Murray
Reg. No. 22,980

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

Ingvar M. FERBY et al. Atty. Docket No.: 100564-00064

Application Number: 09/889,592 Group Art Unit: Not Yet Assigned

Filed: August 2, 2001 Examiner: Not Yet Assigned

For: PROTEIN WITH CELL PROLIFERATION AND CELL DIVISION MODULATING ACTIVITY AND DNA ENCODING SUCH PROTEIN

STATEMENT UNDER 37 C.F.R. § 1.821

Commissioner of Patents
Washington, D.C. 20231

October 31, 2001

Sir or Madam:

In accordance with 37 C.F.R. § 1.821, applicant hereby submits the Sequence Listing for the above-referenced application in paper copy and computer readable form.

The name of the file on the computer readable form is 100564-00064.txt. The computer readable form and the paper copy are the same, and no new matter has been added.

In the event that this paper is not considered timely filed, applicant hereby petitions for an appropriate extension of time. If necessary, please charge any additional

amounts or credit any overpayments to Direct Deposit Account Number 01-2300.

Respectfully Submitted,

ARENT FOX KINTNER PLOTKIN & KAHN, PLLC

D. Daniel Dzara, II
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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

Ingvar M. FERBY et al.

Group Art Unit: Unknown

Application No.: Not Yet Assigned

Examiner: Unknown

Filed: Concurrently herewith

Attorney Dkt. No.: 100564-00064

For: PROTEIN WITH CELL PROLIFERATION AND CELL DIVISION MODULATING ACTIVITY AND DNA ENCODING SUCH PROTEIN

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Date: August 2, 2001

Sir:

Prior to initial examination of the application, please amend the above-identified application as follows:

IN THE CLAIMS:

Please amend claims 3, 4, 6-10, 13, 14 and 16 as follows:

3. (Amended) Expression vector, characterized in that it contains a DNA sequence according to claim 1.

4. (Amended) Protein characterized in that it is encoded by a DNA sequence according to claim 1.

6. (Amended) Protein according to claim 4 characterized in that it shows an oocyte maturation inducing activity and/or a cell division modulating activity.

7. (Amended) Protein according to claim 4, characterized in that it contains deletions, substitutions and/or additions of amino acids that do not substantially affect its activity.

8. (Amended) Protein according to claim 4, wherein a second protein is fused to build a fusion protein.

9. (Amended) Use of a protein according to claim 4, for inducing oocyte maturation and/or modulating cell division and/or differentiation and/or proliferation.

10. (Amended) Pharmaceutical composition containing as active agent a protein according to claim 5.

13. (Amended) Use of a protein according to claim 4, as a diagnostic marker for cell proliferation and/or cell differentiation.

14. (Amended) Use of a protein according to claim 4 as a target for the identification of drugs that modulate cell cycle progression and/or cell proliferation and/or cell differentiation.

16. (Amended) Use of DNA sequence according to claim 1 or a part thereof as diagnostic marker for cell proliferation and/or cell differentiation for hybridization experiments to determine the amount of homologous nucleic acid sequences.

REMARKS

Claims 1-21 are pending in this application. By this Amendment, claims 3, 4, 6-10, 13, 14 and 16 are amended to delete multiple dependency. No new matter is contained in the amendments.

Please charge any fee deficiency or credit any overpayment to Deposit Account No. 01-2300.

Respectfully submitted,

Robert B. Murray
Registration No. 22,980

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RBM/gck

MARKED-UP VERSION OF ORIGINAL CLAIMS

3. (Amended) Expression vector, characterized in that it contains a DNA sequence according to [any one of claims 1 or 2] claim 1.

4. (Amended) Protein characterized in that it is encoded by a DNA sequence according to [any one of claims 1 or 2] claim 1.

6. (Amended) Protein according to claim 4 [or 5] characterized in that it shows an oocyte maturation inducing activity and/or a cell division modulating activity.

7. (Amended) Protein according to [any one of claims 4 to 6] claim 4, characterized in that it contains deletions, substitutions and/or additions of amino acids that do not substantially affect its activity.

8. (Amended) Protein according to [any one of claims 4 to 7] claim 4, wherein a second protein is fused to build a fusion protein.

9. (Amended) Use of a protein according to [any one of claims 4 to 8] claim 4, for inducing oocyte maturation and/or modulating cell division and/or differentiation and/or proliferation.

10. (Amended) Pharmaceutical composition containing as active agent a protein according to [any one of claims 5 to 8] claim 5.

13. (Amended) Use of a protein according to [any one of claims 4 to 8] claim 4, as a diagnostic marker for cell proliferation and/or cell differentiation.

14. (Amended) Use of a protein according to [claims 4 to 8] claim 4 as a target for the identification of drugs that modulate cell cycle progression and/or cell proliferation and/or cell differentiation.

16. (Amended) Use of DNA sequence according to [any one of claims 1 or 2] claim 1 or a part thereof as diagnostic marker for cell proliferation and/or cell differentiation for hybridization experiments to determine the amount of homologous nucleic acid sequences.

19595P WO/BBcl
EMBL

New claim 1

1. A DNA sequence,

characterized in that it contains:

(a) a sequence as shown in SEQ ID NO.1 or 2,

(b) a sequence which encodes the same protein as (a) but is degenerate as a result of the genetic code,

(c) a sequence hybridizing under stringent conditions to the sequences of (a) and/or (b),

(d) a genomic sequence consisting of the sequence according to (a) or (b) and further containing one or more introns,

(e) a sequence which codes for a protein with at the most up to 5% of the amino acid content of the protein according to SEQ ID NO.3 or 4 of deletions, substitutions and/or additions of amino acids and having the same or a very similar activity.

19. Feb. 2001

PCT/EP00/00877
EMBL

New claims

1. A DNA sequence,
characterized in that it encodes a protein that is capable of inducing oocyte maturation and/or modulating cell division and contains:
   (a) a sequence as shown in SEQ ID NO.1 or 2,
   (b) a sequence which encodes the same protein as (a) but is degenerate as a result of the genetic code,
   (c) a sequence hybridizing under stringent conditions to the sequences of (a) and/or (b),
   (d) a sequence according to (a), (b) or (c), wherein this sequences contain one or more introns,
   (e) a sequence which differs from (a), (b), (c) or (d) due to its origin from a different species, but encodes a protein with the same or a very similar activity.

2. A DNA sequence according to claim 1,
characterized in that it further contains expression control sequences operably linked to the coding DNA sequence.

3. Expression vector,
characterized in that it contains a DNA sequence according to anyone of claims 1 or 2.

4. Protein
characterized in that it is encoded by a DNA sequence according to anyone of claims 1 or 2.

5. Protein according to claim 4,
characterized in that it contains an amino acid as shown in SEQ ID NO.3 or 4.

6. Protein according to claim 4 or 5,
characterized in that it shows an oocyte maturation inducing activity and/or a cell division modulating activity.

7. Protein according to anyone of claims 4 to 6,
characterized in that it contains deletions, substitutions and/or additions of amino acids that do not substantially affect its activity.

8. Protein according to anyone of claims 4 to 7,
wherein a second protein is fused to build a fusion protein.

9. Use of a protein according to anyone of claims 4 to 8 for inducing oocyte maturation and/or modulating cell division and/or differentiation and/or proliferation.

10. Pharmaceutical composition containing as active agent a protein according to anyone of claims 5 to 8.

11. Pharmaceutical composition according to claim 10, containing the protein in combination with a pharmaceutically acceptable carrier or adjuvant.

12. Use of a pharmaceutical composition according to claim 10 or 11 for modulating cell proliferation, cell differentiation, or for fertility treatments.

13. Use of a protein according to anyone of claims 4 to 8 as a diagnostic marker for cell proliferation and/or cell differentiation.

14. Use of a protein according to claims 4 to 8 as a target for the identification of drugs that modulate cell cycle progression and/or cell proliferation and/or cell differentiation.

15. Use according to claim 14 for the development of pharmaceuticals for

SEQ ID NO. 1

ls 26 cDNA

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   1    TTA AAC AGG ACT TGC AGC TCC AGT GTA GGT TTT TTC AGA AGC TCC GCC CCA ATG CTG TAT
  61    TTT TTT ATT ATT CCA GGA GGC TAT AAA GAG AGC AGA CAA AGG AAG TAG GCG GAG TTC CTG
 121    TTT ATC GCC ATT TGG CCA GTG GTG GCT AAG CGC CTG AAG GTG GCT GCT TCC TTT GCT CAG
 181    ATC AAC CCT CGG GCC GGT GTC CCC CTT TCT ACA ATG AGG CAC ATG CAG AGT GTA ACC CGG
                                                M   R   H   M   Q   S   V   T   R    9
 241    GCC AGC TCC ATT TGT GGC AGC GGG GTG AAG CAG GTC ATT GGC AAG GGG CAT CCG CAC GCC
        A   S   S   I   C   G   S   G   V   K   Q   V   I   G   K   G   H   P   H   A   29
 301    CGG GTT GTT GGA GCG CGC AAG GCG CAA ATC CCT GAG AGA GAG GAG TTG TCA GTC AAA CCC
        R   V   V   G   A   R   K   A   Q   I   P   E   R   E   E   L   S   V   K   P   49
 361    AAA ATG GTG CGA AAT ACC CAT CTC AAT CTA CAG CCC CAG GAG CGC CAG GCC TTC TAC AGG
        K   M   V   R   N   T   H   L   N   L   Q   P   Q   E   R   Q   A   F   Y   R   69
 421    CTC CTA GAA AAT GAG CAG ATT CAG GAA TTC CTT TCT ATG GAC TCC TGT CTA AGG ATT TCC
        L   L   E   N   E   Q   I   Q   E   F   L   S   M   D   S   C   L   R   I   S   89
 481    GAC AAG TAT CTC ATA GCA ATG GTT CTA GCA TAT TTT AAG CGG GCA GCG GGC CTC TAC ACC
        D   K   Y   L   I   A   M   V   L   A   Y   F   K   R   A   A   G   L   Y   T   109
 541    AGC GAG TAC ACA ACC ATG AAT TTC TTT GTT GCC CTG TAT CTG GCT AAT GAC ATG GAG GAA
        S   E   Y   T   T   M   N   F   F   V   A   L   Y   L   A   N   D   M   E   E   129
 601    GAT GAA GAA GAC TAT AAA TAT GAA ATC TTC CCC TGG GCA CTA GGA GAC TCG TGG CGT GAG
        D   E   E   D   Y   K   Y   E   I   F   P   W   A   L   G   D   S   W   R   E   149
 661    CTT TTC CCA CAA TTT TTG CGT CTC CGG GAC GAC TTC TGG GCT AAA ATG AAC TAC CGA GCA
        L   F   P   Q   F   L   R   L   R   D   D   F   W   A   K   M   N   Y   R   A   169
 721    GTT GTT AgT CGA AGG TGC TGT GAT GAG GTA ATG TCC AAA GAT CCC ACT CAT TGG GCC TGG
        V   V   S   R   R   C   C   D   E   V   M   S   K   D   P   T   H   W   A   W   189
 781    CTG AGA GAT CGC CCC ATG CAT CAC AGC GGG GCC ATG CGT GGT TAC CTT AGA AAC GAG GAC
        L   R   D   R   P   M   H   H   S   G   A   M   R   G   Y   L   R   N   E   D   209
 841    GAC TTT TTC CCC CGG GGT CCA GGC CTT ACA CCA GCC AGC TGT ACA CTT TGC CAT AAA GCA
        D   F   F   P   R   G   P   G   L   T   P   A   S   C   T   L   C   H   K   A   229
 901    GGT GTC TGT GAC TCT GGT GGG GTC TCC CAC AAC AAC TCT TCC TCT CCA GAA CAA GAG ATT
        G   V   C   D   S   G   G   V   S   H   N   N   S   S   S   P   E   Q   E   I   249
 961    TTT CAC TAC ACC AAT AGG GAG TGG TCC CAG GAG CTT CTC ATG TTG CCC CCT GAG CTG TTG
        F   H   Y   T   N   R   E   W   S   Q   E   L   L   M   L   P   P   E   L   L   269
1021    CTG GAT CCC GAG TGT ACT CAT GAC TTA CAC ATT CTC CAG GAG CCA TTG GTT GGA TTA GAG
        L   D   P   E   C   T   H   D   L   H   I   L   Q   E   P   L   V   G   L   E   289
1081    CCA GAT GGG ACG GCG CTG GAA TGG CAC CAC CTT TAG TAG CCG ATT GTC TCC TCC GAG CTT
        P   D   G   T   A   L   E   W   H   H   L   *   *                           300
1141    TTA TTC TTC TCT ACT CAC AAG CTC AGC ACT TAT TCT CTC CTC CTA AGG ACT TGT CAA TGT
1201    TCA GAC TTA ATT GAA ATG GGA GAA GTG AAT ATT CCG ACG GAT GTA GAG CGG GAA TAT GTG
1261    CCC AGA GAA AGT GTT TTG AGT CTG TAT AAA CCG TTG CTT TGT AAA TAA ATA TAT AAA TGT
1321    TCT CTG TGC TGG TCA CTA ATA AAG ATC AGG TAA AAT CAC TTT CAG GTG TAA TTT AAT AGT
1381    ATG TAT GTA GAG TCT TTA ATT CAG CTC TCC ACC AAA TAG TAA CTT GTC ATC ACT GAA CCT
1441    TTG CTT AAC TAC ACT TTT ATT ATT CTG CAC ACA AAT ATT CTG AAG ATC AGA CCG TTC TGT
1501    TTT CAG ATG GGT TGA AAA TAT TAA ACT CAA CAG AAT TCC TGT GGT GTA ATG TAA ATG CAA
1561    AGA TCG ATT AGA CTA
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SEQ ID NO. 2

ls 27 cDNA

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1     GCT GTA TTT TAC TTT CTT TCA GGA GGC TAT AAA GAC AAC AGA CAG GGG AGG TAG GCA GAG
61    TTC CTG TTC ATC ACC ATT CTT TGG CCG TTG GTG GCT AGG CGC CTG AAG GTG GCT GCT ACC
121   TTT GCT CAG ATC AAC CCT CGG TTC GTT GTC CCC CTT TCT ACA ATG AGG CAT ATG CAG AGT
                                                      M   R   H   M   Q   S   6
181   GCA ACC CGG GCC ACC TTA GTT TGT GGC AGC GGG GTA AAG CAG ATC ATT GCC AAG GGA CAT
        A   T   R   A   T   L   V   C   G   S   G   V   K   Q   I   I   A   K   G   H  26
241   CCG AAT ACC CGG GTT TTT GGA GCG CGC AAG GCG AAA ATC CCT GAG AGA GAG GTG CTA GCA
        P   N   T   R   V   F   G   A   R   K   A   K   I   P   E   R   E   V   L   A  46
301   GCC AAA CCC AAG ATC ACG CGC ATT ACA CAT CTC AAT CTA CAA CCC CAG GAG CGC CAG GCC
        A   K   P   K   I   T   R   I   T   H   L   N   L   Q   P   Q   E   R   Q   A  66
361   TTT TAC AGG CTC CTA GAA AAT GAG CTG ATT CAG GAA TTT CTT TCT ATG GAC TCC TGT CTA
        F   Y   R   L   L   E   N   E   L   I   Q   E   F   L   S   M   D   S   C   L  86
421   AAG ATT TCA GAC AAG TAT CTC ATA GCA ATG GTT CTA GCA TAT TTT AAG CGG GCG GGC CTC
        K   I   S   D   K   Y   L   I   A   M   V   L   A   Y   F   K   R   A   G   L  106
481   TAC ACC AGC GAG TAC ACA ACC ATG AAT TTC TTT GTT GCT CTG TAT CTG GCT AAT GAC ATG
        Y   T   S   E   Y   T   T   M   N   F   F   V   A   L   Y   L   A   N   D   M  126
541   GAG GAA GAT GAA GAA GAC TAT AAA TAT GAA ATC TTC CCC TGG GCA CTA GGA GAT TCA TGG
        E   E   D   E   E   D   Y   K   Y   E   I   F   P   W   A   L   G   D   S   W  146
601   CGT GAG TTT TTC CCA CAA TTT TTA CGT CTC CGG GAC GAC TTC TGG GCT AAA ATG AAC TAC
        R   E   F   F   P   Q   F   L   R   L   R   D   D   F   W   A   K   M   N   Y  166
661   CGA GCA GTT GTT AGC CGA AGA TGT TGT GAT GAG GTA ATG GCG AAA GAT CCC ACT CAT TGG
        R   A   V   V   S   R   R   C   C   D   E   V   M   A   K   D   P   T   H   W  186
721   GCC TGG CTC AGA GAT CGT CCT ATT CAT CAT AGT GGG GCC CTG CGT GGT TAC CTC AGA AAT
        A   W   L   R   D   R   P   I   H   H   S   G   A   L   R   G   Y   L   R   N  206
781   GAG GAT GAC TTT TTC CCT CGG GGT CCA GGC CTT ACA CCA GCC AGC TGT GCA CTT TGC CAT
        E   D   D   F   F   P   R   G   P   G   L   T   P   A   S   C   A   L   C   H  226
841   AAA GCA AGT GTC TGT GAC TCT GGT GGG GTG TCC CAT GAC AAC TCT TCT CCA GAA CAA GAG
        K   A   S   V   C   D   S   G   G   V   S   H   D   N   S   S   P   E   Q   E  246
901   ATT TTT CAC TAC ACC AAT AGG GAG TGG TCC CAG GAA CTT CTC ATC TTG CCA CCT GAA CTG
        I   F   H   Y   T   N   R   E   W   S   Q   E   L   L   I   L   P   P   E   L  266
961   TTA TTG GAT CCG GAG TCT ACT TAT GAC ATC CAC ATT TTC CAG GAA CCG TTG GTT GGA TTA
        L   L   D   P   E   S   T   Y   D   I   H   I   F   Q   E   P   L   V   G   L  286
1321  GAG CCA GAT GGG GCA GCC TTG GAA TGG CAC CAC CTT TAG CAC CAT GTC ATC TCT GTG CTT
        E   P   D   G   A   A   L   E   W   H   H   L   *                           298
1081  TCA TTC TTC TCT AAT CCA CGA GCT CAA GAA GCA CTT AAC CTC TCC TAA GCA CTT GCC CAT
1141  GTC CCT ATT CAG ACT AAT GAA TTA AAT GGG AGA GGT GAC TAT TGC CAT AAA GGG AAG GAT
1201  GCC ACT TAG AGT GGA GAA TAA TAC TTG CCA AAA ATG GTG TTT GGG TCT GTT TAA ACT GTT
1261  GCT ATT TCA GTT GCC TTG TAA ATA AAT AAG TAT AAA AAT GTA TGC TCT GTG CCG GTT GCT
1321  AAT AAA AAA AAA ATC TGG TAT CAA AAA AAA AAA AAA A
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SEQ ID NO.3

											M	R	H	M	Q	S	V	T	R	9
A	S	S	I	C	G	S	G	V	K	Q	V	I	G	K	G	H	P	H	A	29
R	V	V	G	A	R	K	A	Q	I	P	E	R	E	E	L	S	V	K	P	49
K	M	V	R	N	T	H	L	N	L	Q	P	Q	E	R	Q	A	F	Y	R	69
L	L	E	N	E	Q	I	Q	E	F	L	S	M	D	S	C	L	R	I	S	89
D	K	Y	L	I	A	M	V	L	A	Y	F	K	R	A	A	G	L	Y	T	109
S	E	Y	T	T	M	N	F	F	V	A	L	Y	L	A	N	D	M	E	E	129
D	E	E	D	Y	K	Y	E	I	F	P	W	A	L	G	D	S	W	R	E	149
L	F	P	Q	F	L	R	L	R	D	D	F	W	A	K	M	N	Y	R	A	169
V	V	S	R	R	C	C	D	E	V	M	S	K	D	P	T	H	W	A	W	189
L	R	D	R	P	M	H	H	S	G	A	M	R	G	Y	L	R	N	E	D	209
D	F	F	P	R	G	P	G	L	T	P	A	S	C	T	L	C	H	K	A	229
G	V	C	D	S	G	G	V	S	H	N	N	S	S	S	P	E	Q	E	I	249
F	H	Y	T	N	R	E	W	S	Q	E	L	L	M	L	P	P	E	L	L	269
L	D	P	E	C	T	H	D	L	H	I	L	Q	E	P	L	V	G	L	E	289
P	D	G	T	A	L	E	W	H	H	L	*	*								300

SEQ ID NO. 4

														M	R	H	M	Q	S	6
A	T	R	A	T	L	V	C	G	S	G	V	K	Q	I	I	A	K	G	H	26
P	N	T	R	V	F	G	A	R	K	A	K	I	P	E	R	E	V	L	A	46
A	K	P	K	I	T	R	I	T	H	L	N	L	Q	P	Q	E	R	Q	A	66
F	Y	R	L	L	E	N	E	L	I	Q	E	F	L	S	M	D	S	C	L	86
K	I	S	D	K	Y	L	I	A	M	V	L	A	Y	F	K	R	A	G	L	106
Y	T	S	E	Y	T	T	M	N	F	F	V	A	L	Y	L	A	N	D	M	126
E	E	D	E	E	D	Y	K	Y	E	I	F	P	W	A	L	G	D	S	W	146
R	E	F	F	P	Q	F	L	R	L	R	D	D	F	W	A	K	M	N	Y	166
R	A	V	V	S	R	R	C	C	D	E	V	M	A	K	D	P	T	H	W	186
A	W	L	R	D	R	P	I	H	H	S	G	A	L	R	G	Y	L	R	N	206
E	D	D	F	F	P	R	G	P	G	L	T	P	A	S	C	A	L	C	H	226
K	A	S	V	C	D	S	G	G	V	S	H	D	N	S	S	P	E	Q	E	246
I	F	H	Y	T	N	R	E	W	S	Q	E	L	L	I	L	P	P	E	L	266
L	L	D	P	E	S	T	Y	D	I	H	I	F	Q	E	P	L	V	G	L	286
E	P	D	G	A	A	L	E	W	H	H	L	*								298

Protein with cell proliferation and cell division modulating activity and DNA encoding such protein

Specification

The present invention relates to DNA sequences, expression vectors containing such DNA sequences, proteins encoded thereby, the use of these proteins for inducing oocyte maturation or modulating cell division and in a pharmaceutical composition, uses as diagnostic markers or for identifying substances modulating the cell cycle progression and/or cell proliferation and/or differentiation, as well as further applications derived therefrom.

Proteins influencing cell division, proliferation or differentiation are generally of great interest. These substances open up a variety of possible uses which can be of interest for several applications depending on the specificity of these proteins. Usually, drugs which make use of the effect of suitable proteins to control or prevent pathological situations can also be derived therefrom. In general, newly found proteins which can be produced recombinantly are therefore received with great interest. They do not only have potential pharmaceutical effects themselves but can often also be used as diagnostic means or as means for developing secondary pharmaceutical agents.

With the current systematical elucidation of the sequences of the human genome (human genome project), many sequences are found which obviously code for proteins. In most cases, though, the function of these proteins is completely unknown, so that it cannot be foreseen which possible uses such products might have.

The object underlying the present invention was to detect sequences coding for proteins influencing the cell cycle, cell division and cell proliferation. It was a further object of the present invention to produce corresponding proteins.

In accordance with the present invention this object was accomplished by providing a DNA sequence characterized in that it contains:

(a) a sequence as shown in SEQ ID NO.1 or 2,

(b) a sequence which encodes the same protein as (a) but is degenerate as a result of the genetic code,

(c) a sequence hybridizing under stringent conditions to the sequences of (a) and/or (b),

(d) a genomic sequence containing the sequence of (a), (b) or (c) and further containing one or more introns,

(e) a sequence which differs from (a), (b), (c) or (d) due to its origin from a different species.

In order to find proteins involved in cell cycle activation, a Xenopus oocyte cDNA was prepared and cloned in expression vectors. The primary library was subdivided into pools and plasmid DNA was purified from the pools and in vitro transcribed to obtain mRNAs. The mRNA pools were injected into stage VI oocytes which were incubated to allow for protein expression. Pools which upon microinjection in oocytes were capable of inducing oocyte maturation on their own or of strongly accelerating progesterone-induced maturation were subdivided into smaller pools and reinjected until single positive clones were isolated. Following this approach, out of a huge number of mRNA pools two specific sequences corresponding to SEQ ID NOs.1 and 2 were isolated. These sequences do not correspond by DNA hybridization experiments to any known proteins inducing oocyte maturation, including protein kinase Mos, the protein phosphatase cdc25 and several A and B type cyclins. The mRNA prepared from the two isolated clones containing SEQ ID NO.1 or 2 was used for protein expression and

the obtained proteins were found capable of potently inducing oocyte maturation also in the absence of progesterone stimulation. The obtained DNA sequence data as shown in SEQ ID NOs.1 and 2 demonstrate that these two clones contain open reading frames coding for related proteins.

The present invention comprises the sequences shown in SEQ ID NOs.1 and 2 which, however, may also contain certain deviations. In particular, the present invention covers deviations which are present in the DNA only but which, owing to the diversity of the genetic code, encode the same protein as SEQ ID NO.1 or 2. Furthermore, the present invention comprises sequences which hybridize under stringent conditions with SEQ ID NO.1 or 2, or sequences deviating therefrom as set out under (b). The present invention also comprehends the corresponding genomic sequences of the cDNA sequences of SEQ ID NO.1 or 2, or of sequences deviating therefrom as set out under (b) or (c). Such genomic sequences may contain one or more introns which are cleaved off during translation and processing and thus do not influence the finally encoded protein.

Still further, the present invention comprises sequences which deviate from those of (a), (b), (c) or (d) owing to their origin from a different species.

Very often, highly conserved DNAs coding for proteins which have the same activities in different species, such as mouse or human, show only slight differences. In most cases, deviations occur only in some nucleotides and/or in few amino acids of the coded protein. Hence, by means of the concretely disclosed sequences as of SEQ ID NO.1 or 2, corresponding nucleic acids in other species, which code for proteins with the same or a very similar activity, can easily be found. Such similar sequences are therefore comprised by the present invention, too.

The DNA sequences according to the invention encode proteins capable of inducing oocyte maturation and/or modulating cell division, proliferation and/or differentiation.

The term "modulating" according to the invention is meant to encompass promotion as well as inhibition. Experimental results show that proteins encoded by the DNA according to the invention show in some instances a promoting, in other instances an inhibiting effect on cell division.

In a preferred embodiment of the invention the DNA sequences further contain expression controlled sequences which are operably linked to the coding DNA sequence. Any suitable expression control sequences may be used for the present invention. Particularly preferred sequences are those which allow a favourable control of expression, such as sequences allowing induction of expression or inhibition of expression. Induction or inhibition generally takes place via the binding of a respective inductor or inhibitor molecule to operator sequences. Corresponding expression control sequences are known to the person skilled in the art, the lac operator being an example therefor.

A further subject matter of the present invention is an expression vector containing a DNA sequence according to the invention.

As set out above for the DNA sequences, the expression vector also particularly preferably comprises expression control sequences allowing for specific expression control. Also, sequences that allow for positive selection of transformed host cells are known to the man in the art and are preferably introduced in the expression vectors according to the invention.

A further subject matter of the present invention is a protein encoded by a DNA sequence according to the present invention. As explained above, the protein according to the invention, which preferably contains an amino acid

sequence according to SEQ ID NO.3 or 4, modulates cell proliferation and differentiation. Oocyte maturation is induced by the proteins of the invention whereas expression in some mammalian cell lines seems to inhibit cell division. The particularly preferred proteins of SEQ ID NOs.3 and 4 are capable of inducing oocyte maturation considerably faster than the same amount of injected malE-Mos or progesterone treatment. The entire cell cycle in Xenopus oocytes is extraordinarily strongly activated by the proteins of the invention. Only low amounts of the protein of the invention are required to stimulate oocyte maturation.

The protein according to the present invention may have deletions, substitutions and/or additions of amino acids in regions which do not affect the activity. However, the activity of the protein must not be considerably impaired thereby. Further, it is preferred that at the most up to 5 % of the amino acid content of the protein of the invention has deletions, substitutions and/or additions of amino acids. It is not difficult for the skilled artisan to find out which regions may contain deletions, substitutions or additions. Corresponding changes can be made in the nucleic acids, followed by expression and an activity test. By means of site-directed mutagenesis manifold variants can easily be produced and expressed. The person of skill in the art can easily simultaneously test a multitude of such mutants for their activity (high-throughput screening), whereby as a prerequisite at least half the activity of the proteins shown in SEQ ID NO.3 or 4 has to be retained. By means of computer-aided conformation studies the regions of the protein which are less probably involved in the activity of the protein can be determined. Particularly in such regions can mutations be made.

A still further subject matter of the present invention is the use of the protein according to the invention for modulating oocyte maturation and/or promoting cell division, cell proliferation or cell differentiation.

As a further subject matter of the present invention a pharmaceutical composition may be formulated on the basis of this possible use. The pharmaceutical composition according to the invention contains as active agent a protein according to the invention which in particular contains the amino acids as of SEQ ID NO.3 or 4 or sequences derived therefrom which may exhibit the aforementioned mutations, deletions or substitutions.

Preferably, the pharmaceutical composition contains the protein in combination with a pharmaceutically acceptable carrier or adjuvant.

The pharmaceutical composition according to the invention may be used for all pathological situations in which it is desired to modulate cell proliferation, cell differentiation or cell maturation. Examples for such applications are the promotion of growth and maturation of specific cell types, e.g. ovarian cells, so that the pharmaceutical composition of the invention is in particular also useful and suitable for fertility treatments.

The protein according to the invention can further be used as diagnostic marker for cell proliferation and/or cell differentiation. The amount of said protein contained in an organism can be correlated to the cell proliferation or differentiation rate. As soon as a basic value has been determined, the amount of this protein present in, e.g., different development stages of cells can be determined, thus showing the particular development status.

A further possible use of the proteins according to the invention lies in their capability of acting as a target for the identification of drugs modulating cell cycle progression and/or cell proliferation and/or cell differentiation. By means of these proteins a multitude of substances can be tested for their modulation capability. To this end, a system is provided which comprises cells susceptible to the proteins of the invention, proteins according to the invention and a substance which is to be examined as to its modulating activity. It can then be determined whether the activity of the protein to

modulate cell proliferation and/or differentiation is weakened or even prevented by said substance. Using high-throughput screening (HTPS), such experiments can be carried out for a multitude of substances simultaneously. A particularly preferable use for identifying substances modulating cell proliferation and/or differentiation lies in the development of drugs for the treatment of cancer or other pathological situations with uncontrolled cell proliferation.

Especially carcinoma grow by uncontrolled cell division, and the inhibition thereof is highly desired. Substances allowing to block such division can be found using the protein of the invention. Even the protein itself might be applicable for inhibiting uncontrolled cell growth in cancers.

Another subject matter of the present invention is the use of the DNA sequences according to the invention as diagnostic marker for the cell proliferation and/or cell differentiation status, whereby the amount of homologous nucleic acids present in the cell is determined by hybridization experiments. Of particular interest is the amount of mRNAs hybridizing to the DNA according to the invention. For this purpose, preferably the DNA sequence according to the invention or a part thereof is labelled, so that after performance of the hybridization experiment the formed double strands may be easily detected. Particularly preferably, a single-stranded DNA sequence should be used corresponding to the antisense strand of the DNA according to the invention. Using such an antisense strand DNA which is complementary to the mRNA, the actual amount of formed protein can be determined on a nucleic acid basis.

In combination with the figures the following examples are to further illustrate the present invention.

Fig. 1 shows a sequence comparison of proteins Is26 and Is27.

Fig. 2 shows the result of experiments with injection of small amounts of recombinant malE-Is26 protein (10 ng) into Xenopus oocytes, leading to oocyte maturation considerably faster than the same amount of injected malE-Mos or a progesterone treatment. Fig. 2 also shows the same experiment where cycloheximide preincubation blocked malE-Mos-induced GVBD (germinal vesicle breakdown) but had no effect on Is26-triggered oocyte maturation.

Fig. 3 shows the malE-Is26-induced activation of MAP kinase and cdc2/cyclin B in an immunoblot and by direct measurement of the in vitro kinase activity using MBP and histone H1 as substrates for MAP kinase and cdc2/cyclin.

Fig. 4 also shows an immunoblot using anti-cdc2 antibodies and an in vitro histone H1 kinase assay.

Fig. 5 shows a pull-down experiment using rabbit reticulocyte lysates which demonstrates that Is26 can directly bind to B-type cyclins.

Example 1

To identify novel proteins implicated in cell cycle activation in Xenopus oocytes, an expression cloning strategy was used where a Xenopus oocyte cDNA library was constructed in the FTX5 expression vector. The primary library was subdivided into pools of 150-200 colonies and plasmid DNA was purified from the pools and in vitro transcribed to obtain mRNAs. The mRNA pools were injected into stage VI oocytes which were incubated for 30 - 36 hours to allow protein expression from the injected mRNAs prior to stimulation with progesterone. Finally, those pools which upon microinjection in oocytes were capable either of inducing oocyte maturation

on their own or of strongly accelerating progesterone-induced maturation were subdivided into smaller pools and reinjected until single positive clones were isolated.

Using this approach, out of 105 mRNA pools injected in oocytes two clones were isolated which did not correspond by DNA hybridization experiments to proteins that are known to induce oocyte maturation including the protein kinase Mos, the protein phosphatase cdc25 and several A and B type cyclins. The mRNAs prepared from the two isolated clones, which were referred to as Is26 and Is27, were capable of potently inducing oocyte maturation in the absence of progesterone stimulation. DNA sequencing showed that these two clones contained open reading frames that encode for related proteins and were fused in frame to the C-terminus of the myc tag in the FTX5 vector.

Full-length Is26 and Is27 cDNAs were cloned from a λ ZAP Xenopus oocyte cDNA library using as probes the two cDNAs isolated in the expression screening. The Is26 clone was 1574 base pairs and encoded for a protein of 300 amino acids (SEQ ID NO.1), whilst Is27 was 1357 base pairs in length and encoded for a protein of 298 amino acids (SEQ ID NO.2). Both clones contained stop codons upstream of the first ATG and in the same frame (underlined in SEQ ID NO.1 and 2). The predicted Is26 and Is27 proteins were 91% identical (Fig. 1). When the Is26 and Is27 sequences were tested against DNA and protein sequence data bases, no significant homologies (Blast search) could be detected, suggesting that Is26/Is27 belong to a novel protein family. Programmes were also tested which were designed to identify conserved protein motifs (for example Prosite), but again there was no clue as to the kind of activity that the Is26/Is27 proteins may have. Thus, based on the lack of sequence homology, the Is26/Is27 proteins do not appear to have any known catalytic activity (protein kinase, phosphatase,...).

Example 2

To investigate the function of the Is26/Is27 proteins, the two cDNAs were cloned in the bacterial expression vector pMalc2 downstream of the malE gene. The fusion proteins malE-Is26 and malE-Is27 were expressed in and purified from E. coli. Since Is26 and Is27 are very similar in sequence and probably correspond to pseudoalleles, which are quite common in Xenopus, one concentrated on the characterization of Is26 and then confirmed the results obtained using Is27. It was found that the injection into Xenopus oocytes of small amounts of recombinant malE-Is26 protein (10 ng) was capable of inducing oocyte maturation considerably fater than the same amount of injected malE-Mos or than progesterone treatment (Fig. 2). This experiment using the fusion protein confirmed the results observed with mRNA in vitro transcribed from the Is26 cDNA clone regarding the potency of this novel protein to induce cell cycle activation in Xenopus oocytes. It was also found that injection of only 0.5 ng of malE-Is26 per oocyte was still acapable of inducing oocyte maturation. The availability of purified malE-Is26 protein also allowed to test the capability of Is26 to induce oocyte maturation in the presence of protein synthesis inhibitors. Preincubation of the oocytes with cycloheximide totally blocked progesterone-induced maturation, consistent with the known essential requirement for translation of maternal mRNAs stored in the oocytes for progesterone to induce maturation. In the same experiment, cycloheximide preincubation also blocked malE-Mos-induced GVBD but it had no effect on Is26-triggered oocyte maturation (Fig. 2).

Example 3

To further characterize the activity of the Is26 protein, the kinetics of activation of MAP kinase (MAPK) and cdc2/cyclin B (MPF) in oocytes induced to mature by malE-Is26 were investigated. MAPK and MPF are normally activated during oocyte maturation and their activation can be

detected in oocyte lysates either by immunoblot with anti-MAPK and anti-cdc2 antibodies or by direct measurement of the in vitro kinase activity using MBP and histone H1 as substrates for MAPK and MPF, respectively (Fig. 3). As expected from previous work, we observed that progesterone treatment activates both MAPK and MPF at about the same time, whereas malE-Mos injection activates MAPK well before MPF activation. Interestingly, injection of malE-Is26 rapidly activates MPF somewhat before MAPK. Moreover, MPF appears to be transiently activated by Is26, but the significance of this observation is unclear. In cycloheximide-treated oocytes, the Is26-induced activation of MAPK is very much reduced whereas the activation of MPF is apparently unaffected. This result indicates that the effect of Is26 is more related to MPF activation than to MAPK activation. As expected, cycloheximide totally blocked progesterone-induced activation of both MPF and MAPK, whereas in the case of Mos only MPF but not MAPK activation was compromised by cycloheximide.

Example 4

The observation that Is26 can consistently induce oocyte maturation and the activation of MPF independently of new protein synthesis is quite remarkable as only proteins that act very late in the activation pathways, such as cyclins (cdc2 binding and activating subunits) or direct cdc2/cyclinB activators such as the cdc25 phosphatase have been shown to have this strong effect. In order ot address whether Is26 can directly associate with and/or modify the acitvity of cdc2/cyclin B complexes, pull-down experiments were performed. For this purpose, extracts prepared from insect cells infected with cdc2-expressing baculovirus were incubaed with either malE-Is26 bound to amylose beads or the equivalent amount of cyclin B bound to nickel beads. After extensive washing, the proteins that remained bound to the beads were analyzed by immunoblot using anti-cdc2 antibodies and in vitro histone H1 kinase assay. We found that Is26 bound to cdc2 with almost the same efficiency of cyclin B (Fig. 4). When the

cyclin B pull-down was done in the presence of a 2-fold molar excess of soluble malE-Is26, we observed a reduction in the amount of cdc2 bound to cyclin B which also correlated with the expected decrease in the kinase activity of the complexes. This suggests that Is26 may compete with cyclin B to bind to cdc2. By immunoblot using anti-malE antibodies we confirmed that cyclin B was capable of binding Is26 in the presence of cdc2. Our results indicate that Is26 can strongly bind to cdc2 and probably also to cyclin B, but we do not know whether Is26 can complex to cdc2/cyclin B.

The interaction between Is26 and cdc2 was confirmed using ^{35}S-methionine-labelled cdc2 prepared by coupled transcription/translation in rabbit reticulocyte lysates. We also confirmed in pull-down experiments with rabbit reticulocyte lysates that Is26 can directly bind to B-type cyclins (Fig. 5).

the treatment of cancer or other pathological situations with uncontrolled cell proliferation.

16. Use of a DNA sequence according to anyone of claims 1 or 2 or a part thereof as diagnostic marker for cell proliferation and/or cell differentiation for hybridization expriments to determine the amount of homologous nucleic acid sequences.

```
Is 26      1  MRHMQSVTRASSICGSGVKQVIGKGHPHARVVGARKAQIPEREE
Is 27      1  MRHMQSATRATLVCGSGVKQIIAKGHPNTRVFGARKAKIPEREV

Is 26     45  LSVKPKMVRNTHLNLQPQERQAFYRLLENEQIQEFLSMDSCLRI
Is 27     45  LAAKPKITRITHLNLQPQERQAFYRLLENELIQEFLSMDSCLKI

Is 26     89  SDKYLIAMVLAYFKRAAGLYTSEYTTMNFFVALYLANDMEEDEE
Is 27     89  SDKYLIAMVLAYFKRA-GLYTSEYTTMNFFVALYLANDMEEDEE

Is 26    133  DYKYEIFPWALGDSWRELFPQFLRLRDDFWAKMNYRAVVSRRCC
Is 27    132  DYKYEIFPWALGDSWREFFPQFLRLRDDFWAKMNYRAVVSRRCC

Is 26    177  DEVMSKDPTHWAWLRDRPMHHSGAMRGYLRNEDDFFPRGPGLTP
Is 27    176  DEVMAKDPTHWAWLRDRPIHHSGALRGYLRNEDDFFPRGPGLTP

Is       221  ASCTLCHKAGVCDSGGVSHNNSSSPEQEIFHYTNREWSQELLML
Is 27    220  ASCALCHKASVCDSGGVSHDNSS-PEQEIFHYTNREWSQELLIL

Is 26    265  PPELLLDPECTHDLHILQEPLVGLEPDGTALEWHHL
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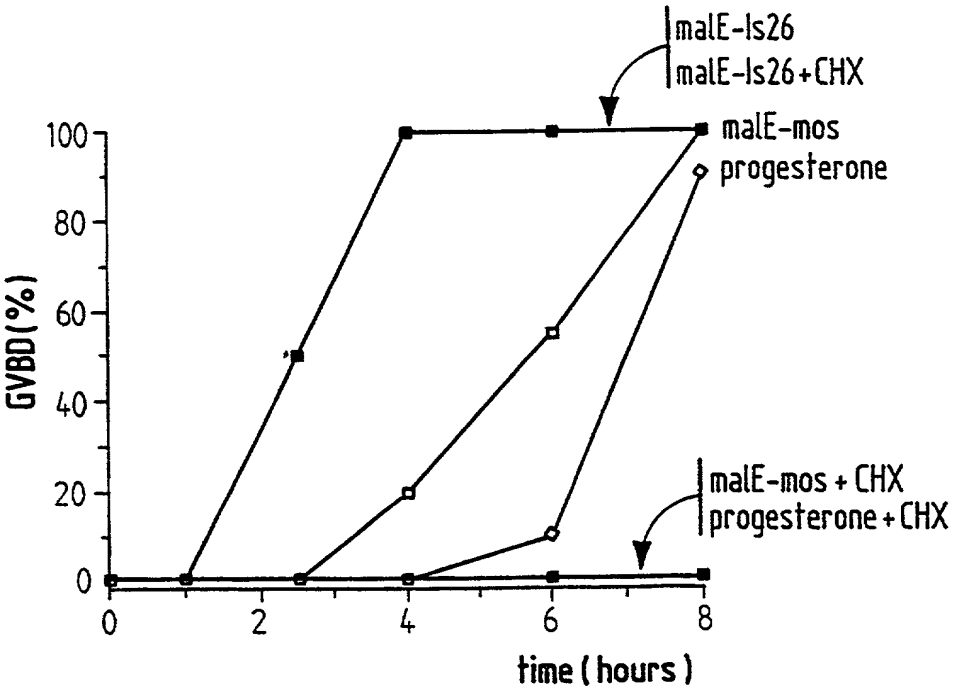
Fig. 1


malE-Is26
malE-Is26+CHX
malE-mos
progesterone
malE-mos + CHX
progesterone + CHX
GVBD (%)
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80
60
40
20
0
0
2
4
6
8
time (hours)

Fig. 2

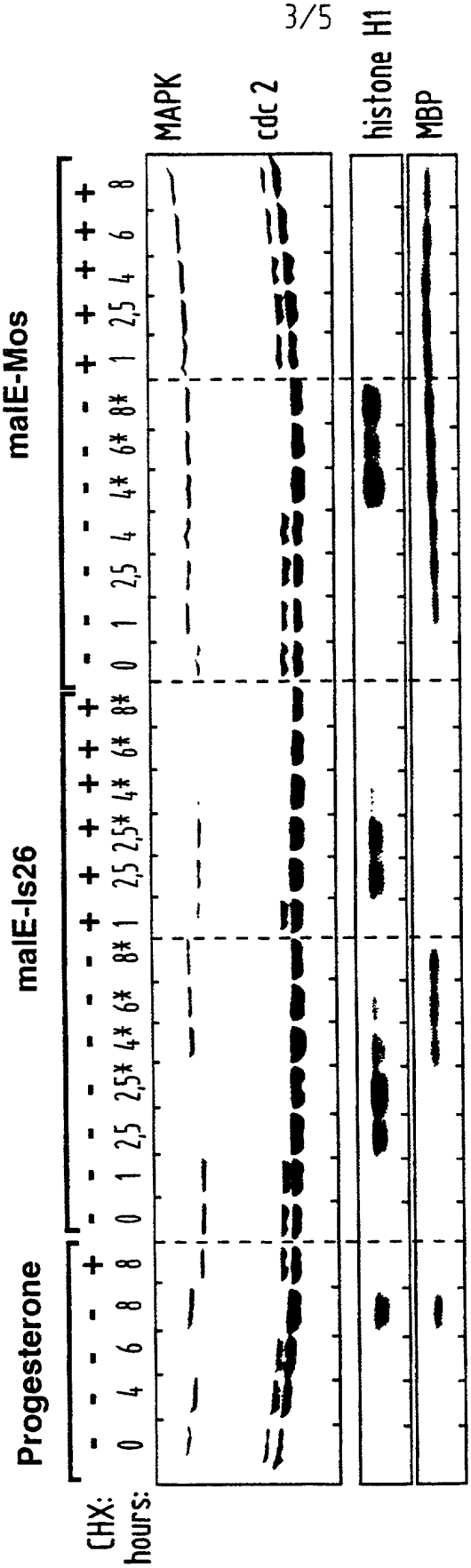
MAPK
cdc 2
histone H1
MBP
malE-Mos
malE-Is26
Progesterone
CHX:
hours:
- - - - - + - - - - - - + + + + + + + + - - - - - - + + + + + +
0 4 6 8 8 0 1 2,5 2,5* 4* 6* 8* 1 2,5 2,5* 4* 6* 8* 0 1 2,5 4 4* 6* 8* 1 2,5 4 6 8

Fig. 3

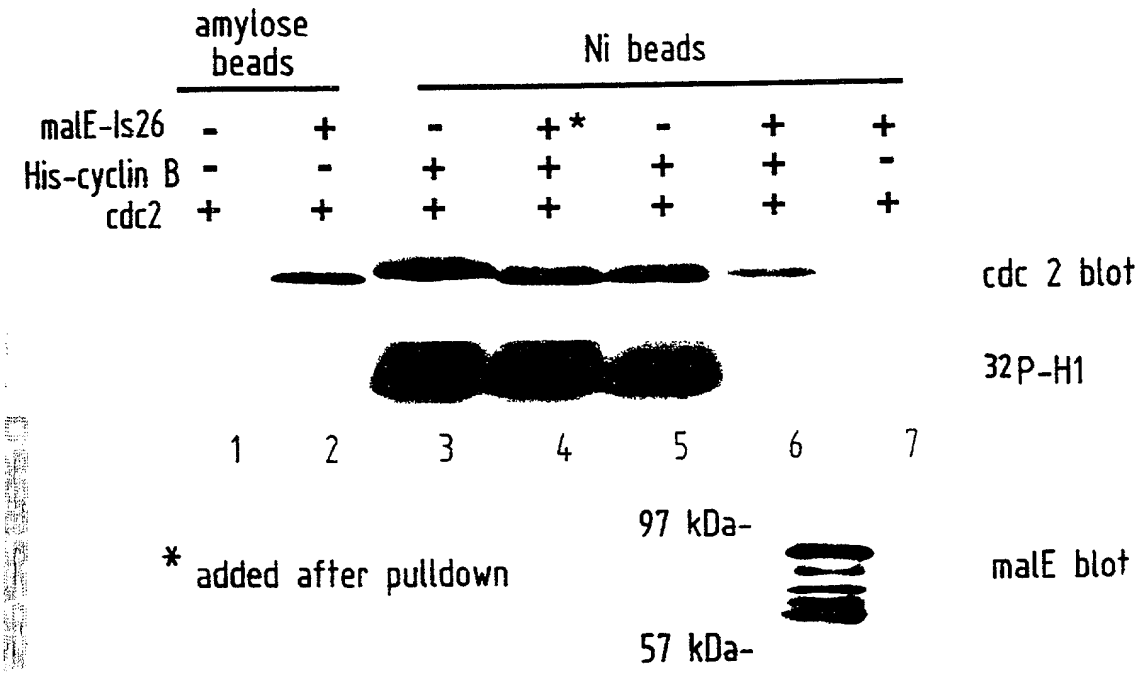
amylose beads
Ni beads
malE-Is26 - + - +* - + +
His-cyclin B - - + + + + -
cdc2 + + + + + + +
cdc 2 blot
32P-H1
1 2 3 4 5 6 7
97 kDa-
57 kDa-
malE blot
* added after pulldown

Fig. 4

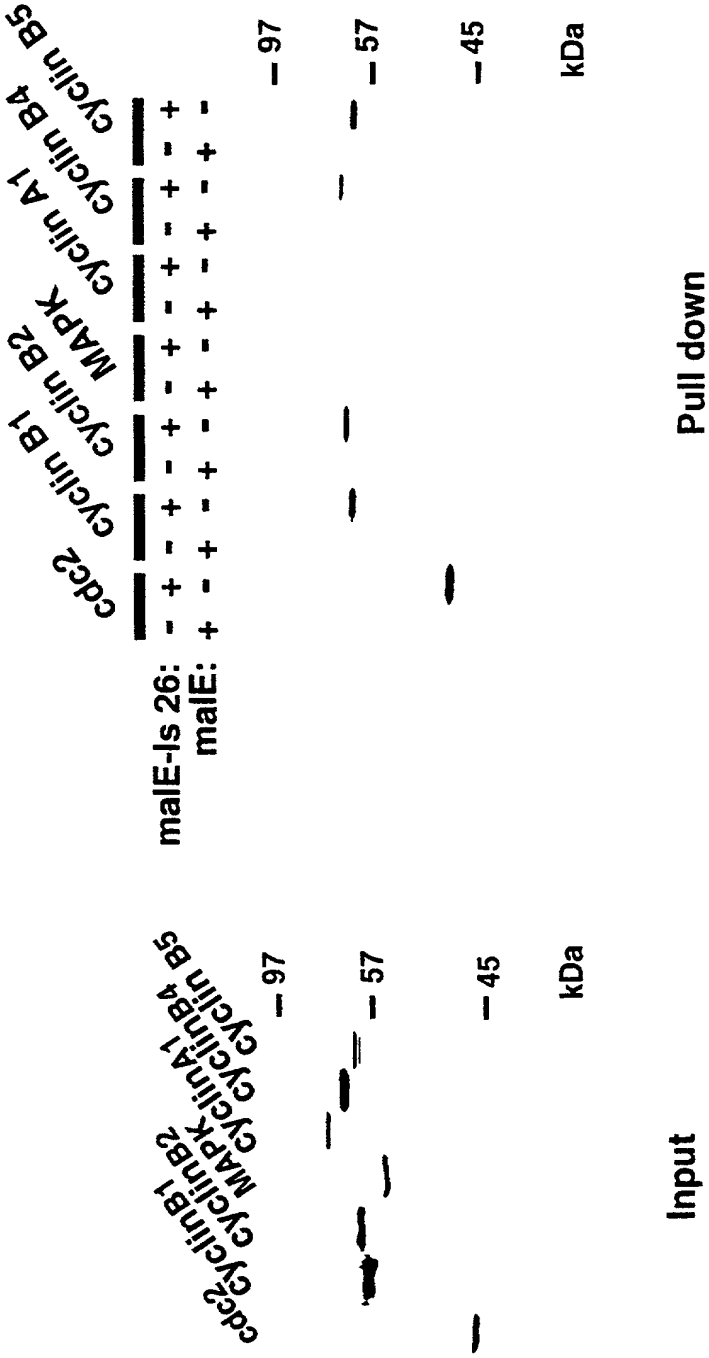
Pull down
Input
kDa
97
57
45
malE:
malE-Is 26:
cdc2
cyclin B1
cyclin B2
MAPK
cyclin A1
cyclin B4
cyclin B5
cdc2 cyclinB1
cyclinB2
MAPK cyclinA1
cyclinB4
cyclin B5

Fig. 5

19595P US-WO

Docket No. _____

ARENT FOX KINTNER PLOTKIN & KAHN, PLLC
Nikaido, Marmelstein, Murray & Oram Intellectual Property Group

Declaration For U.S. Patent Application

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
(Insert Title) Protein with cell proliferation and cell division modulating activity and DNA encoding such protein
the specification of which is attached hereto unless the following box is checked:

XX was filed on February 03, 2000 as PCT International Application Number PCT/EP00/00877 and was amended on Feb 19, 2001 and May 09, 2001

and/or was filed on _____ as United States Application Number _____ and was amended on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56.
I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International Application having a filing date before that of the application(s) for which priority is claimed:

(List prior foreign applications. See note A on back of this page)	(Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed
	99 102 172.6	EP	Feb 03, 1999	☒ Yes ☐ No
				☐ Yes ☐ No
				☐ Yes ☐ No

I hereby claim the benefit under 35 U.S.C. §119(c) of any United States provisional application(s) listed below.

(Application Number) _____ (Filing Date) _____

(Application Number) _____ (Filing Date) _____

(See Note B on back of this page) ☐ See attached list for additional prior foreign or provisional applications.

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or §365(c) of any PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) (U.S. or PCT) in the manner provided by the first paragraph of 35, U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(List prior U.S. Applications or PCT International applications designating the U.S.)	(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

And I hereby appoint as principal attorneys: Robert B. Murray, Reg. No. 22,980; Charles M. Marmelstein, Reg. No. 25,895; George E. Oram, Jr., Reg. No. 27,931; Douglas H. Goldhush. Reg. No. 33.125; David T. Nikaido, Reg. No. 22,663; Monica Chin Kitts, Reg. No. 36,105; Richard J. Berman, Reg. No. 39,107; King L. Wong, Reg. No. 37,500; James A. Poulos, III, Reg. No. 31,714; Patrick D. Muir, Reg. No. 37,403; Murat Ozgu, Reg. No. 44,275; Bradley D. Goldizen, Reg. No. 43,637, N. Alexander Nolte, Reg. No. 45,689 and Robert K. Carpenter, Reg. No. 34,794. 14

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

(See Note C on back of this page)

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Residence _____
Citizenship _____
Post Office Address _____

Full name of fourth joint inventor, if any _____
Inventor's signature _____ Date
Residence _____
Citizenship _____
Post Office Address _____

Full name of fifth joint inventor, if any _____
Inventor's signature _____ Date
Residence _____
Citizenship _____
Post Office Address _____

Full name of sixth joint inventor, if any _____
Inventor's signature _____ Date
Residence _____
Citizenship _____
Post Office Address _____

Full name of seventh joint inventor, if any _____
Inventor's signature _____ Date
Residence _____
Citizenship _____
Post Office Address _____

Full name of eighth joint inventor, if any _____
Inventor's signature _____ Date
Residence _____
Citizenship _____
Post Office Address _____

Full name of ninth joint inventor, if any _____
Inventor's signature _____ Date
Residence _____
Citizenship _____
Post Office Address _____

SEQUENCE LISTING

<110> Europäisches Laboratorium für Molekularbiologie

<120> Protein with cell proliferation and cell division modulating activity and DNA encoding such protein

<130> 19595PWO

<140> PCT/EP00/00877
<141> 2000-02-03

<150> EP 99102172.6
<151> 1999-02-03

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<170> PatentIn Ver. 2.1

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acc cgg gcc agc tcc att tgt ggc agc ggg gtg aag cag gtc att ggc 282
Thr Arg Ala Ser Ser Ile Cys Gly Ser Gly Val Lys Gln Val Ile Gly
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Lys Gly His Pro His Ala Arg Val Val Gly Ala Arg Lys Ala Gln Ile
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Ile Ser Asp Lys Tyr Leu Ile Ala Met Val Leu Ala Tyr Phe Lys Arg
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Ala Ala Gly Leu Tyr Thr Ser Glu Tyr Thr Thr Met Asn Phe Phe Val
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Ala Leu Tyr Leu Ala Asn Asp Met Glu Glu Asp Glu Glu Asp Tyr Lys
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Tyr Glu Ile Phe Pro Trp Ala Leu Gly Asp Ser Trp Arg Glu Leu Phe
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Arg Ala Val Val Ser Arg Arg Cys Cys Asp Glu Val Met Ser Lys Asp
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ccc act cat tgg gcc tgg ctg aga gat cgc ccc atg cat cac agc ggg   810
Pro Thr His Trp Ala Trp Leu Arg Asp Arg Pro Met His His Ser Gly
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gcc atg cgt ggt tac ctt aga aac gag gac gac ttt ttc ccc cgg ggt   858
Ala Met Arg Gly Tyr Leu Arg Asn Glu Asp Asp Phe Phe Pro Arg Gly
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Pro Gly Leu Thr Pro Ala Ser Cys Thr Leu Cys His Lys Ala Gly Val
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Leu Pro Pro Glu Leu Leu Leu Asp Pro Glu Cys Thr His Asp Leu His
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Glu Trp His His Leu
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Ser Met Asp Ser Cys Leu Arg Ile Ser Asp Lys Tyr Leu Ile Ala Met
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Val Leu Ala Tyr Phe Lys Arg Ala Ala Gly Leu Tyr Thr Ser Glu Tyr
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Thr Thr Met Asn Phe Phe Val Ala Leu Tyr Leu Ala Asn Asp Met Glu
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Glu Asp Glu Glu Asp Tyr Lys Tyr Glu Ile Phe Pro Trp Ala Leu Gly
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Asp Ser Trp Arg Glu Leu Phe Pro Gln Phe Leu Arg Leu Arg Asp Asp
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Phe Trp Ala Lys Met Asn Tyr Arg Ala Val Val Ser Arg Arg Cys Cys
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Asp Glu Val Met Ser Lys Asp Pro Thr His Trp Ala Trp Leu Arg Asp
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Arg Pro Met His His Ser Gly Ala Met Arg Gly Tyr Leu Arg Asn Glu
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Asp Asp Phe Phe Pro Arg Gly Pro Gly Leu Thr Pro Ala Ser Cys Thr
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Leu Cys His Lys Ala Gly Val Cys Asp Ser Gly Gly Val Ser His Asn
225                 230                 235                 240

Asn Ser Ser Ser Pro Glu Gln Glu Ile Phe His Tyr Thr Asn Arg Glu
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Trp Ser Gln Glu Leu Leu Met Leu Pro Pro Glu Leu Leu Leu Asp Pro
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                                              Met Arg His Met
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Gln Ser Ala Thr Arg Ala Thr Leu Val Cys Gly Ser Gly Val Lys Gln
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Ala Lys Ile Pro Glu Arg Glu Val Leu Ala Ala Lys Pro Lys Ile Thr
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agg ctc cta gaa aat gag ctg att cag gaa ttt ctt tct atg gac tcc   414
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tgt cta aag att tca gac aag tat ctc ata gca atg gtt cta gca tat   462
Cys Leu Lys Ile Ser Asp Lys Tyr Leu Ile Ala Met Val Leu Ala Tyr
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tat aaa tat gaa atc ttc ccc tgg gca cta gga gat tca tgg cgt gag   606
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aac tac cga gca gtt gtt agc cga aga tgt tgt gat gag gta atg gcg   702
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aaa gat ccc act cat tgg gcc tgg ctc aga gat cgt cct att cat cat   750
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agt ggg gcc ctg cgt ggt tac ctc aga aat gag gat gac ttt ttc cct   798
Ser Gly Ala Leu Arg Gly Tyr Leu Arg Asn Glu Asp Asp Phe Phe Pro
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cgg ggt cca ggc ctt aca cca gcc agc tgt gca ctt tgc cat aaa gca   846
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Ser Val Cys Asp Ser Gly Gly Val Ser His Asp Asn Ser Ser Pro Glu
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atc ttg cca cct gaa ctg tta ttg gat ccg gag tct act tat gac atc   990
Ile Leu Pro Pro Glu Leu Leu Leu Asp Pro Glu Ser Thr Tyr Asp Ile
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cac att ttc cag gaa ccg ttg gtt gga tta gag cca gat ggg gca gcc   1038
His Ile Phe Gln Glu Pro Leu Val Gly Leu Glu Pro Asp Gly Ala Ala
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Leu Glu Trp His His Leu
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35 40 45

Pro Lys Ile Thr Arg Ile Thr His Leu Asn Leu Gln Pro Gln Glu Arg
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Gln Ala Phe Tyr Arg Leu Leu Glu Asn Glu Leu Ile Gln Glu Phe Leu
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Ser Met Asp Ser Cys Leu Lys Ile Ser Asp Lys Tyr Leu Ile Ala Met
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Val Leu Ala Tyr Phe Lys Arg Ala Gly Leu Tyr Thr Ser Glu Tyr Thr
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Thr Met Asn Phe Phe Val Ala Leu Tyr Leu Ala Asn Asp Met Glu Glu
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Thr Tyr Asp Ile His Ile Phe Gln Glu Pro Leu Val Gly Leu Glu Pro
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Asp Gly Ala Ala Leu Glu Trp His His Leu
290 295